

**Novel Neuroprotective Therapy with NeuroHeal by Autophagy Induction for
Damaged Neonatal Motoneurons**

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SUPPLEMENTARY INFORMATION

- **Supplementary Tables**

Table S1. Primary antibodies for immunohistochemistry

Antibody name	Reference
rabbit anti-Iba1	1:1000, Wako, ref 1919741
rabbit anti-SIRT1	1:100, Millipore, ref 07-131
rabbit anti-H3K9Ac	1:50, Millipore, ref 06-942
rabbit anti-p53-K373Ac	1:500, Millipore, ref 06-916
rabbit anti-cleaved Casp3	1:200; Cell Signaling, ref 96615
rabbit anti-LAMP1	1:200, Antibodies Online, ref 3187789
mouse anti-ATG5	1:200, Nanotool, ref 0263-100
mouse anti-p62	1:100; BD Transduction Laboratories, ref 610833
rabbit anti-phospho-FOXO3a (Ser253)	pFOXO3a; 1:500, Abcam, ref ab 31109-100
rabbit anti-FOXO3a	Novus Biologicals, 1:200, ref NBP2-16521
rabbit anti-phospho-S6 kinase (Thr389)	pP70S6k; 1:1000, Antibodies Online, ref 863186
rabbit anti-PARP1/2	1:200; Santa Cruz, ref sc-7150
anti- β -III-tubulin	1:500, Covance, ref MMS-435P/801202
anti SMI32	1:1000; Biolegend, ref 801701

Table S2. Primary antibodies for Western Blot

Antibody name	Reference
rabbit anti-PARP1/2	1:500; Santa Cruz Biotechnology, ref sc-7150
rabbit anti-phospho-Ulk1 (Ser 555)	pUlk1; 1:1000; Millipore, ref ABC124
mouse anti-Atg5	1:1000; Nanotools, ref 0263-100
rabbit anti-LC3	1:1000; Abcam, ref ab48394
rabbit anti-pFOXO3a	1:1000, Abcam, ref ab31109-100
rabbit anti-FOXO3a	Novus Biologicals, 1:500, ref NBP2-16521
rabbit anti-phospho-AKT	pAKT; 1:1000; Cell Signaling, ref 4060

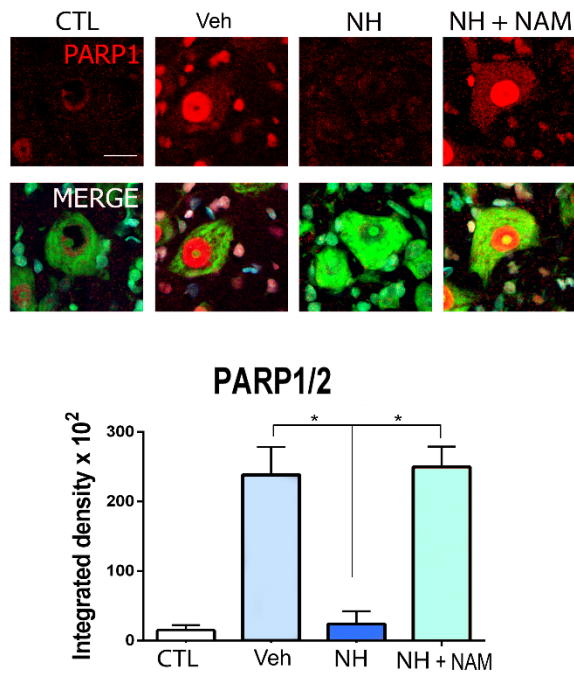
rabbit anti-AKT	1:1000; Cell Signaling, ref 4691
Mouse anti- β -actin	1:3000, Sigma Aldrich, ref A5316

Table S3. Primers used for RT-PCR

Primer Name	Sequence (5' to 3')
<i>rBNIP3-F</i>	CAGAGCGGCGAGGAGAACCTGCAG (24)
<i>rBNIP3-R</i>	GCTGCTCCCATTTCCATTGCTGAAG (25)
<i>rBeclin-F</i>	GGTAGCTTTTCTGGACTGTGTGCAGCAG (28)
<i>rBeclin-R</i>	GTCTTCAATCTTGCCTTTCTCCACGTCC (28)
<i>rLC3-F</i>	ACCCTCCCTGCATGCAGCTGTCC (23)
<i>rLC3-R</i>	ACCAGGGACATGACGACGTACACAACC (27)
<i>rGADD45-R</i>	TGAGGGTGAAATGGATCTG (19)
<i>rGADD45-F</i>	GAAGATCGAAAGGATGGAC (19)
<i>rBax2-F</i>	CTGCAGAGGATGATTGCTGA (20)
<i>rBax2-R</i>	GATCAGCTCGGGCACTTTAG (20)
<i>rActin-F</i>	TCAGGTCATCACTATCGG (18)
<i>rActin-R</i>	CAGTAATCTCCTTCTGCATC (20)

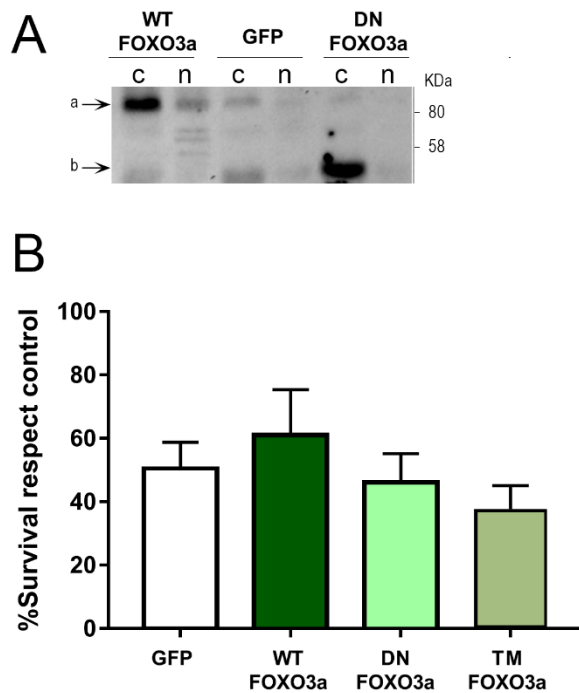
- [Supplementary Figures and Legends](#)

Supplementary Figure S1



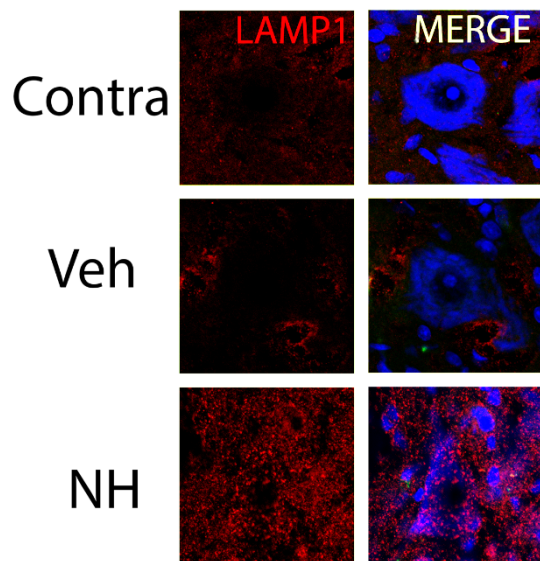
Supplementary Figure S1. Top, Representative confocal microphotographs of the immunolabelling of PARP1/2 (red), and FluoroNissl (green), and DAPI (blue) in merged pictures of MNs from different experimental conditions at 10 dpi. Scale bar 20 μ m. **Bottom,** Quantification of the integrated density of PARP1/2 in nuclei of MNs (n=4 per group, ANOVA, post hoc Bonferroni, *p<0.05).

Supplementary Figure S2



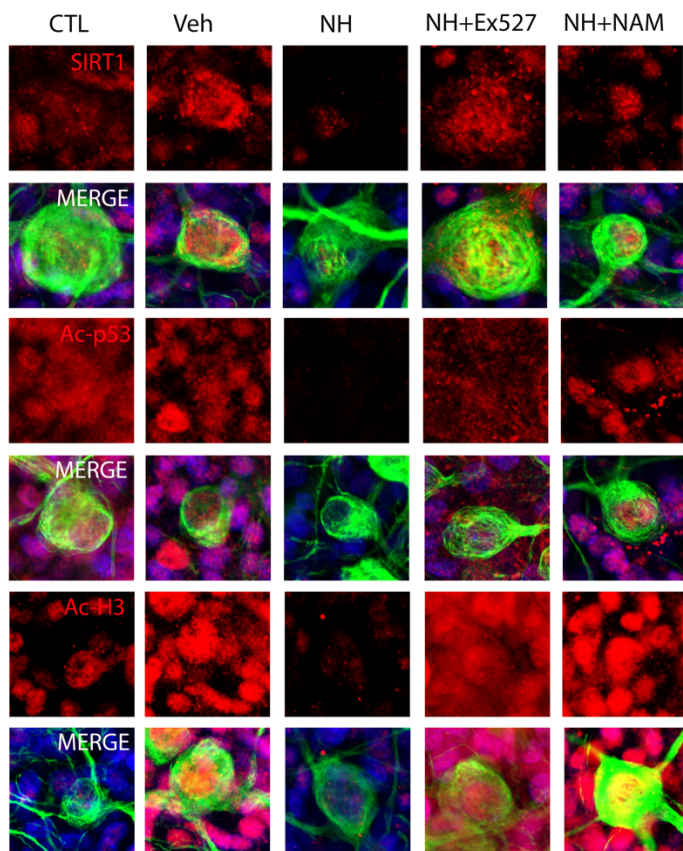
Supplementary Figure S2. (A) Representative images from two independent experiments of immunoblots to detect FOXO3a (top) or BIM (down) from NSC34 cells either untransfected (CTL) or nucleofected with either GFP, double negative (DN) or nonphosphorylatable triple mutant (TM) plasmids. Note that FOXO3a corresponding band is normally detected above 80 Kda (band a) except when expression of the deleted form of DN mutant (<50KDa, band b). (B) Graph showing the percentage survival (\pm SEM) of transfected NSC-34 cells with respect to cells transfected with control vector analysed at 4 DIV.

Supplementary Figure S3



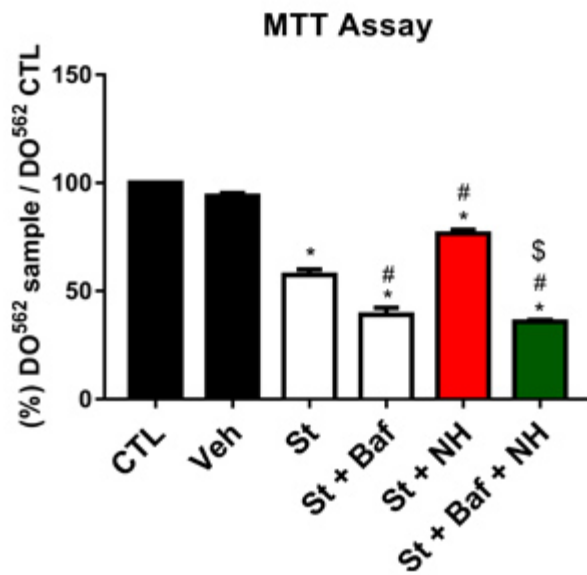
Supplementary Figure S3. Confocal microphotographs of samples stained for LAMP1 (red), and counterstained with FluoroNissl (blue) from a representative contralateral side and injured side of vehicle-treated and NH-treated injured animals at 10 dpi. Scale bar 25 μm .

Supplementary Figure S4



Supplementary Figure S4. Confocal microphotographs of SOC cells immunolabelled for SIRT1, p53Ac, or H3K9Ac (red), SMI3-32 (green), and DAPI (blue) at 15 DIV after the treatment with vehicle, NeuroHeal (NH), or NH+SIRT1 inhibitors. Scale bar 25 μ m.

Supplementary Figure S5



Supplementary Figure S5. Analysis of cell survival using MTT assay in different conditions: 100 μ M Bafilomycin A1 (a V-ATPase inhibitor, Baf) to apoptotic cells induced by staurosporine (St) and treated with NeuroHeal (NH). Ctrl, control; veh1, 0.0002% DMSO; veh2, 0.01 % etOH. * $p < 0.05$ vs Control; # $p < 0.05$ vs St; \$ $p < 0.05$ vs St+NH.

- **Supplementary Material**

Extra Immunoblot Membranes for Fig 2B_ Casp3

